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Effect of flushing and estrus synchronization in Kenguri ewes

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Abstract
The study was conducted to know the efficacy of different synchronization protocols in postpartum anoestrous Kenguri ewes in non-breeding season. Thirty healthy ewes of 60 days postpartum were selected and divided in to 5 groups, each containing 6 animals (n=6). All the five groups were fed with maize grains (250 gm/ewe/day) for 15 days prior to synchronization protocol as flushing protocol. G-I served as control group with only maize feeding without treatments. After 15 days of Maize feeding, 4 treatment groups received CIDR intravaginal device (0.3 gm of progesterone) left intravaginal for 12 days. After 12 days, during the removal of CIDR, G-II and G-IV animals received I/M inj. PGF2 α @ 125 µg /ewe whereas G-III and G-V received I/M inj. PMSG @ 500 IU/ewe. Upon exhibition of estrus and natural mating, G-IV and G-V animals received I/M inj. hCG @ 500 IU/ewe. Estrus response (%) was 33.33, 33.33, 66.66, 50.00 and 66.66 and conception rate (%) was 50.00, 50.00, 50.00, 66.66 and 75.00 in G-I, G-II, G-III, G-IV and G-V respectively. In conclusion, hCG treated animals had better conception rate in postpartum anoestrous Kenguri ewes.

Keywords: Kenguri, flushing, estrus synchronization

Introduction
Sheep is an important livestock of India and are believed to have been one of the first mammals to be domesticated and are known to have been closely associated with man from a very early date. They contribute greatly to the agrarian economy, especially in the arid/semi-arid and mountainous areas where crop and /or dairy farming are not economical. They play an important role in the livelihood of a large percentage of small and marginal farmers and landless labourers engaged in sheep rearing. A number of rural-based industries use wool and sheep skins as raw material. Sheep manure is an important source of soil fertility, especially in southern states. Main factor in sheeprerarıng is its seasonality, which limits their fecundity and following economic gains.

Patterns of reproductive activity in the adult ewes are dominated by two distinct rhythms. The first of them is a 16 to 17 day long estrous cycle. The other is an annual rhythm of ovarian cyclicity characterized by a season-dependent cessation (anoestrus) and restoration (breeding season) of ovulatory ovarian cycles (Goodman, 1994; Gordon, 1996) [5, 6]. In the longer days of spring, there is a break in the reproductive period, whereas the shorter days of autumn are associated with the onset of estrus (Dogan and Nur, 2006) [7]. Thus, reproductive seasonality is an important factor that limits the productivity of small ruminants (Zarazaga *et al.*, 2003) [10].

Reproductive seasonality can be manipulated by using hormonal treatments (Atsan *et al.*, 2007) [9]. There are several methods for improvement of reproduction in ewes, which often aim to increase the proportion of ewes having multiple ovulations, and thereby increase lambing rate (Akoz *et al.*, 2006) [8]. In small ruminants, estrus synchronization is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin F2α or by extending the cycle artificially with exogenous progesterone or more potent progestogen (Jainudeen *et al.*, 2000; Kusina *et al.*, 2000) [11, 12]. As prostaglandin treatment is limited to the breeding season, different protocols of estrus synchronization using progestin's have been introduced (Rosado *et al.*, 1998) [13].

The most common protocol for estrus synchronization in sheep is based on manipulating the endocrine environment of ewes with intention of increasing ovulation rate using exogenous supplementation of gonadotrophins (Moore and Rowson, 1960) [14] or 5 progestogen/ progesterone treatment in the form of intravaginal implants (Sponges/CIDR) which are used

either for 12-14 days (long period) or for 5-7days (short period) (Abecia *et al.*, 2011) [15].

In view of this, the present research work was carried out with the following objectives: 1. to know the efficacy of synchronization with different protocols in postpartum anestrous ewes. 2. To study the effect of flushing in postpartum anestrous ewes.

2 Materials and Methods

2.1 Location of The Study: The present study was carried out on Kenguri ewes maintained at Department of Instructional Livestock Farm Complex, Veterinary College, Nandinagar, Bidar and field flocks (private farmers) in and around Bidar District. The study was conducted during the period of March 2018 to May 2018.

2.2 Selection of Animals: Thirty healthy ewes, aged about 2-5 years which have not shown estrus up to 60 days postpartum were selected for this study. Animals were already vaccinated against PPR. Deworming was carried out with Fenbendazole @7.5 mg/kg BW. Animals were allowed to graze during day hours from 10:00 a.m. to 5:00 p.m. and provided clean water ad libitum. These animals were randomly divided into five different groups. Number of animals in each group was six.

2.3 Synchronization Protocols

Animals were divided into 6 groups. Group I (n=6), the group was treated as control. Each animal was fed with 250 g of maize daily (morning) for 15days and later observed for estrus signs without receiving any of the treatment. Group II(n=6) recieved Flushing (maize feeding for 15 D) with CIDR device (for 12 D intra vaginally) followed by Inj PGF2α at CIDR removal. Group III (n=6) recieved similar maize feeding for 15 Days followed by CIDR device kept intravaginally for 12 D followed by Inj PMSG at CIDR removal. Group IV (n=6) Recieved Flushing (maize feeding for 15 D) followed by CIDR device kept intravaginally for 12 Days followed by Inj PGF2α at CIDR removal also Inj hCG upon Natural service. Group V(n=6) Flushing with maize feeding for 15 Days followed by CIDR device kept intravaginally for 12 Days followed by Inj PMSG at CIDR removal along with Inj hCG upon Natural service. The animals were tested for pregnancy and different serum hormonal levels were analyzed to find out best protocol with high pregnancy rate.

2.4 Serum Profiles

Blood samples were collected by jugular vein puncture from all the groups on day -15, -7, day 0, 7, 12 and at detected estrus. Serum samples were harvested by subjecting through centrifugation at 3000 rpm for 10 minutes and using micro pipette serum sample was made into aliquot in duplicate and stored at -20˚ C until analysis. The serum samples were subjected for serum progesterone analysis performed by ELISA (CALBIOTECH Progesterone ELISA kit). Serum glucose and cholesterol analysis was performed as per the assay procedures mentioned in the kits (Swemed Diagnostics, Bengaluru) using auto chemistry blood analyzer (Artos Elita, Swemed Biomedicals, Pvt Ltd, Bengaluru).

2.5 Pregnancy Diagnosis

The lower abdominal region of the ewes was shaved before submitting them for scanning after day 60 of post-mating. The ewes were subjected to pregnancy diagnosis by using trans-abdominal approach of real time B-mode ultrasonography using 5 to 7.5 MHz 45 multi frequency sector array probe. The ewes were diagnosed as pregnant based on the visualization of an enlarged uterine lumen with amniotic fluid, which appeared as an anechoic area near the anechoic zone of urine filled bladder. Pregnancy was also diagnosed on the basis of visualization of echoic embryo within the amniotic cavity, placentomes and fetal skeleton (Ganaie *et al.*, 2009) [16].

2.6 Statistical Analysis

The data obtained was analysed by SAS 9.3 software using one – way ANOVA (Kaps and Lamberson, 2004) [17].

3. Results

In G I , 2 animals responded to protocol thus estrus induction observed was 33.33 % (Table 1), Mean ± SE values for onset of estrus was 204.00 ± 12.00 h, Mean ± SE values for duration of estrus was 24.88 ± 0.88 h and an animal got conceived out of 2 exhibited estrus thus the conception rate was 50.00 % in this group. In G II ,2 animals responded to synchronization protocol thus estrus induction observed in this group was 33.33 % (Table 1), Mean ± SE values for onset of estrus was 61.83 ± 3.17 h , Mean ± SE values for duration of estrus was 30.54 ± 0.79 h and out of 2 estrus exhibited animals, an animal got conceived thus forming the conception rate of 50.00 %. In G III out of 6 animals, 4 animals showed estrus thus estrus induction observed in this group was 66.66 % (Table 1), Mean ± SE values for onset of estrus was 44.25 ± 1.00 h (Table 2 and Fig 2), Mean ± SE values for duration of estrus was 37.12 ± 0.58 h and 2 animals got conceived out of 4 animals exhibited estrus thus the conception rate was 50.00 %. In G IV out of 6 animals, 3 animals exhibited estrus thus estrus induction observed in this group was 50.00 % (Table 1), Mean ± SE values for onset of estrus was 62.53 ± 1.85 h and Mean ± SE values for duration of estrus was 29.03 ± 1.07 h. Out of 3 exhibited estrus animals, 2 animals got conceived in this present study thus the conception rate was 66.66 %. In G V, out of 6 animals 4 animals responded to treatment and exhibited estrus and the estrus induction observed in this group was 66.66 % (Table 1), Mean ± SE values for onset of estrus was 45.15 ± 1.34 h, Mean ± SE values for duration of estrus was 35.39 ± 1.17 h and 3 animals got conceived out of 4 thus the conception rate was 75.00 %.

Hence, among all the groups the animals which received PMSG and hCG had highest conception rate and also estrus response. The control group tough had low conception rate but showed better estrus response.

Also G III animals displayed a better estrus response than G I, II and IV but similar to that of G V (66.66 %) but conception rate was similar to GI & G II (50 %). G IV animals displayed next best result in estrus display (50 %) and conception (50 %).

4. Discussion

Low Estrus induction and conception rate in control group(G I) were found to be low, might be due to adverse environmental effects as this trial was conducted during the month of March-April-May which is known to thermal stress. According to Casu *et al.* (1991) [18] heat stress as adverse effect on ovulation in the ewes and Hooda and Naqvi (1990)

[19] stated that heat stress will be experienced by the ewes according to their quality nutrition. In G II animal's non responsiveness of PGF2α might be the reason of low expression of estrus. PGF2α will be sensitive to act on ovary in the ovulatory season only. However outside the ovulatory season it will not respond (Abecia *et al.*, 2011) [15].

In G IV and V the conception rate was more than the other group animals due to the beneficiary and stimulatory effect of the PMSG and hCG on the follicular cycle.

Hence, use of these hormones judiciously can be helpful in estrus induction in anestrous Ewes with a good conception rate. This can help in increasing the farmer's income from this additional reproduction.

Table 1: Estrus response (%), Onset of Estrus (h), (Mean ± S.E), Duration of Estrus (h), (Mean ± S.E) and Conception rate (%) of control and synchronised groups

Groups	No., of animals (n)	No., of animals induced to estrus	Estrus response (%)	Onset of Estrus (h)	Duration of Estrus (h)	No., of animals conceived (n)	Conception rate (%)
G I	6	2	33.33	204.00 ± 12.00	24.88 ± 0.88	1	50
G II	6	2	33.33	61.83 ± 3.17	30.54 ± 0.79	1	50
G III	6	4	66.66	44.25 ± 1.00	37.12 ± 0.58	2	50
G IV	6	3	50	62.53 ± 1.85	29.03 ± 1.07	2	66.66
G V	6	4	66.66	45.15 ± 1.34	35.39 ± 1.17	3	75

Note: GI: Control (only Flushing), G II: Flushing+ CIDR+ PGF2α, G III: Flushing+ CIDR+ PMSG, G IV: Flushing+ CIDR+ PGF2α + hCG, G V: Flushing+ CIDR+ PMSG+ hCG.

Conclusion

Based on the present research findings, the following conclusions were drawn.

1. Supplementation of 250 gm maize to 60 day postpartum anestrous animals in nonbreeding season has shown the increased blood glucose level but induction of estrus was only 33.33 % with 50.00 % conception rate indicates flushing in non-breeding season is not enough to induce estrus.
2. Use of CIDR and PGF2α in non-breeding season with flushing also not shown any significant change in estrus induction or conception rate in 60 days postpartum anestrous Kenguri ewes indicates limitation of use of PGF2α in acyclic animals in nonbreeding season.
3. Use of PMSG proved its beneficial effect on induction, onset and duration of estrus in postpartum anestrous Kenguri ewes in non-breeding season.
4. In the present study, use of hCG has shown very beneficial effect on conception rate of postpartum Kenguri ewes with the combination of CIDR, PGF2α and PMSG.

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